

## SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF BENZILIC ACID-TYROSINE METAL COMPLEXES

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### ABSTRACT

The antimicrobial drugs occupy a unique niche in the history of medicine. Amino acid complexes are proving to be an efficient antimicrobial drug nowadays. The new Benzilic acid –tyrosine metal complexes were prepared on condensation of Benzilic acid and the amino acid, tyrosine and the synthesized compounds were authenticated by UV-Visible, Fourier Transformer-Infrared (FT-IR) and Gouy Method techniques, Elemental analysis, Molar conductance study. Tyrosine, a non-essential amino acid used widely as a precursor to neurotransmitters. Benzilic acid and its derivatives are of wide interest due to their diverse antimicrobial activities. The synthesized compounds were authenticated by UV-Visible, Fourier Transformer-Infrared (FT-IR) and Gouy Method techniques. The biological activity of the DT ligand and its complexes has been tested for antibacterial effects against *Staphylococcus Aureus* and *Escherichia coli* using well diffusion method. The antibacterial study revealed that compound S4 showed excellent activity against gram positive bacteria *Staphylococcus Aureus*. The S4 complex was found to be more potent than free ligand showing that on complexation the activity of the complexes were altered.<sup>[1]</sup> Certain metal ions possess effective antimicrobial activity; here the Cobalt complex is efficient to act against *Staphylococcus Aureus*.

**KEYWORDS:** BT ligand, Benzilic acid (D), Tyrosine(T), *Staphylococcus Aureus* and *Escherichia coli*.

### 1. INTRODUCTION

Benzilic acid and the substituted Benzilic acid compounds are significant compounds with high potential to act as antimicrobial agents. The inhibiting potential of bacteria against the antibiotics is a major confront to the mankind. Therefore the need of more resistant antibiotics has a crucial role in the research world. A pertinent report by World Health Organization, on resistant antibacterial agents for human medicine enlighten the urge for updating the list of critically important antibacterial agents regularly as new information becomes available, including data on resistant patterns, new and emerging diseases and the development of new drugs.<sup>[2]</sup> Therapeutic values of Benzilic acid derivatives has become the subject of an ongoing research. The anti histamine activity and anti acetylcholine activity of the Benzilic acid and their derivatives have been reported.<sup>[3]</sup> The different substituents in the benzilic acid and its derivatives were synthesized and their antimicrobial activity has been studied.<sup>[4]</sup> Thermally stable fungicides were synthesized from Benzilic acid-amino acid complexes of transition metals as Ni, Cu, Mn and Co.<sup>[5,6]</sup> Antimicrobial activity of cobalt complexes against various bacterial and fungal strains were studied by Subhasish Saha et al.<sup>[7]</sup> Nathmala

and Yadav<sup>[8]</sup> studied antifungal activity of organo tin complexes derived from 2 hydroxy-1-naphthaldehyde and benzaldehyde amino acid. The physical and biological factors have an influence on the antimicrobial activity of compounds in which nature of metal ion plays a crucial role. Andrea<sup>[9]</sup> et al studied the antibacterial properties of the copper and cobalt amino acid complexes. The current study discussed the antibacterial effect of Cobalt complexes of Benzilic acid –tyrosine ligand on the gram positive bacteria *Staphylococcus aureus*. The ligand does not possess any activity but on complexation the activity of the compound have been enhanced.

### 2. MATERIALS AND METHODS

#### 2.1. Materials

Benzilic acid, tyrosine and metal salts were used without purification. The solvents were purified using standard procedure. The melting points of the ligand and the complexes were recorded with the melting point apparatus. The Gouy balance is used to determine the magnetic susceptibility of the complexes. The characterization of the compounds conducted with the aid of elemental analysis, Fourier-transform infrared (FT-IR) and electronic spectra.

## 2.2. Synthesis of Benzilic acid-tyrosine ligand (HBT)

0.1 M solution of Benzilic acid in aqueous ethanol was mixed with 0.1 M ethanolic solution of tyrosine potassium salt and refluxed for 3 hours on water bath. The resulting solution concentrated for few minutes and the ligand crystallizes out and washed with ethanol and dried over anhydrous CaCl<sub>2</sub>. The melting point was found to be 298<sup>0</sup> C.

## 2.3. Synthesis of the metal complexes

The metal complexes were prepared by adding the metal acetate/chloride solution dropwise to the hot ethanolic solution of Benzilic acid –tyrosine (HBT) solution. A pinch of sodium acetate trihydrate was added and was refluxed for 1 hour, and cooled to room temperature. The crystalline precipitate was collected and washed several times with water and dried in dessicator.

## 3. CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

Table 1: Analytical data of ligand (HBT) and its complexes.

Compound	Molecular Weight	Melting point	Colour	yield	$\Omega^{-1}$	$\mu_{\text{eff}}$	M%
C <sub>23</sub> H <sub>19</sub> NO <sub>5</sub> (HBT)	391.43	290 <sup>0</sup>	Pale yellow	80	-	-	-
Cr(BT)(H <sub>2</sub> O) <sub>4</sub>	515.43	>300 <sup>0</sup>	Dark green	65	8.55	1.52	10.08 (10.27)
Fe(BT)(H <sub>2</sub> O) <sub>2</sub> Cl <sub>2</sub>	554.27	>300 <sup>0</sup>	Light brown	70	9.73	3.18	10.07 (10.12)
Co(BT)(H <sub>2</sub> O) <sub>4</sub>	522.36	>300 <sup>0</sup>	Pink brown	60	4.56	4.32	11.28 (11.90)
Ni(BT) <sub>2</sub> (H <sub>2</sub> O)	877.69	>300 <sup>0</sup>	Bluish green	60	4.79	3.38	6.68 (6.40)

### 3.1. Benzilic acid-tyrosine ligand

The FT-IR spectrum of the ligand was recorded in the range 4000-400 cm<sup>-1</sup>. The sharp peak at 3207 cm<sup>-1</sup> may be attributed to N-H stretching vibration. The broad band at 3437 cm<sup>-1</sup> is assigned to the O-H stretching vibration.<sup>[10]</sup> The bands at 1609 and 1416 cm<sup>-1</sup> are assigned to  $\nu$  (COO<sub>asymm</sub>) and  $\nu$  (COO<sub>symm</sub>) respectively. The  $\nu$  (C=O) band is at 1732 cm<sup>-1</sup> and the  $\nu$  (C-O) band is at 1244 cm<sup>-1</sup>. The solid state uv-visible spectrum of the ligand was recorded in the region 200-900 nm. The compound gives peaks at 233,279 and 330 nm in which former peak indicates the  $\pi \rightarrow \pi^*$  transition and the latter may be due to  $n \rightarrow \pi^*$  transition.

### 3.2. Chromium complex of Benzilic acid-tyrosine ligand

The IR of Chromium complex shows a peak at 3205cm<sup>-1</sup> which is assigned to the N-H vibration suggests that NH bond do not involve in the complexation. The disappearance of band at 3437 cm<sup>-1</sup> predicts the involvement of OH group of carboxylic acid in the coordination to the metal ion. The bands at 1491 and 1344 cm<sup>-1</sup> are attributed to  $\nu$  (COO<sub>asymm</sub>) and  $\nu$  (COO<sub>symm</sub>) respectively. The difference between  $\nu$  (COO<sub>asymm</sub>) and  $\nu$  (COO<sub>symm</sub>) is  $\approx 200$  cm<sup>-1</sup> which suggests the monodentate behaviour of the carboxylate anion. The band at 841 cm<sup>-1</sup> assigned to the coordinated water. Peak at 698 cm<sup>-1</sup> is due to the  $\nu$  (M-O) stretching of the complex. The solid state uv-visible spectrum gives peaks at 233,279 and 330 nm in which former peak indicates the  $\pi \rightarrow \pi^*$  transition and the latter may be due to  $n \rightarrow \pi^*$  transition. They exhibits three spin-allowed transitions i.e. <sup>4</sup>A<sub>2g</sub> (F)  $\rightarrow$  <sup>4</sup>T<sub>2g</sub> (F) ( $\nu_1$ ), <sup>4</sup>A<sub>2g</sub> (F)  $\rightarrow$  <sup>4</sup>T<sub>1g</sub> (F) ( $\nu_2$ ), <sup>4</sup>A<sub>2g</sub> (F)  $\rightarrow$  <sup>4</sup>T<sub>1g</sub> (P) ( $\nu_3$ ) at 340 nm, 351 nm and 594 nm respectively suggesting the octahedral stereochemistry of the chromium complexes.

### 3.3. Iron complex of Benzilic acid-tyrosine ligand

The IR of Iron complex shows a peak at 3205 cm<sup>-1</sup> is assigned to the N-H vibration. The bands at 1512 and

1330 cm<sup>-1</sup> are attributed to  $\nu$  (COO<sub>asymm</sub>) and  $\nu$  (COO<sub>symm</sub>) respectively. The difference between  $\nu$  (COO<sub>asymm</sub>) and  $\nu$  (COO<sub>symm</sub>) is  $\approx 200$  cm<sup>-1</sup> which suggests the monodentate behaviour of the carboxylate anion. The band of OH group of carboxylic acid at 3437cm<sup>-1</sup> is absent in the complex suggests the complexation of the group with the metal ion. The band at 840 cm<sup>-1</sup> assigned to the coordinated water. Peak at 649 cm<sup>-1</sup> is due to the  $\nu$  (M-O) stretching of the complex. The solid state uv-visible spectrum gives peaks at 233,279 and 330 nm in which former peak indicates the  $\pi \rightarrow \pi^*$  transition and the latter may be due to  $n \rightarrow \pi^*$  transition. In the current work absorption band of Fe (II) complex are in the range 891 nm assigned to <sup>5</sup>T<sub>2g</sub>  $\rightarrow$  <sup>5</sup>E<sub>g</sub> transition. A strong charge transfer band is observed at 389 nm. From spectral data, an octahedral geometry is proposed for the Fe (II) chelate.

### 3.4. Cobalt complex of Benzilic acid-tyrosine ligand

The IR of chromium complex shows a peak at 2919cm<sup>-1</sup> is assigned to the N-H vibration. The bands at 1466 and 1382 cm<sup>-1</sup> are attributed to  $\nu$  (COO<sub>asymm</sub>) and  $\nu$  (COO<sub>symm</sub>) respectively. The difference between  $\nu$  (COO<sub>asymm</sub>) and  $\nu$  (COO<sub>symm</sub>) is  $\approx 200$  cm<sup>-1</sup> which suggests the monodentate behaviour of the carboxylate anion. The band of OH group of carboxylic acid at 3437cm<sup>-1</sup> is absent in the complex suggests the complexation of the group with the metal ion. The band at 824 cm<sup>-1</sup> assigned to the coordinated water. Peak at 495 cm<sup>-1</sup> is due to the  $\nu$  (M-O) stretching of the complex. The solid state uv-visible spectrum gives peaks at 233,279 and 330 nm in which former peak indicates the  $\pi \rightarrow \pi^*$  transition and the latter may be due to  $n \rightarrow \pi^*$  transition. Two bands present in electronic spectra of Co (II) at  $\approx 881$  and 260 nm. It is assignable to octahedral geometry.

### 3.5. Nickel complex of Benzilic acid-tyrosine ligand

The IR of chromium complex shows a peak at 2971 cm<sup>-1</sup> is assigned to the N-H vibration. The bands at 1466 and

1382  $\text{cm}^{-1}$  are attributed to  $\nu$  ( $\text{COO}_{\text{asym}}$ ) and  $\nu$  ( $\text{COO}_{\text{sym}}$ ) respectively. The difference between  $\nu$  ( $\text{COO}_{\text{asym}}$ ) and  $\nu$  ( $\text{COO}_{\text{sym}}$ ) is  $\approx 200 \text{ cm}^{-1}$  which suggests the monodentate behaviour of the carboxylate anion. The band of OH group of carboxylic acid at  $3437 \text{ cm}^{-1}$  is absent in the complex suggests the complexation of the group with the metal ion. Peak at  $515 \text{ cm}^{-1}$  is due to the  $\nu$  (M-O) stretching of the complex. The solid state uv-visible spectrum gives peaks at 233, 279 and 330 nm in which former peak indicates the  $\pi \rightarrow \pi^*$  transition and the latter may be due to  $n \rightarrow \pi^*$  transition. Nickel chelate exhibits three bands in the region 895 nm, 670 nm and 371 nm. Using energy level diagram these band are assigned to the transition  ${}^3\text{A}_2\text{g}(\text{F}) \rightarrow {}^3\text{T}_2\text{g}(\text{F}) (\nu_1)$ ,  ${}^3\text{A}_2\text{g}(\text{F}) \rightarrow {}^3\text{T}_1\text{g}(\text{F}) (\nu_2)$  and  ${}^3\text{A}_2\text{g}(\text{F}) \rightarrow {}^3\text{T}_1\text{g}(\text{P}) (\nu_3)$  respectively, for an octahedral stereochemistry.

### 3.6. METHOD

#### 3.6.1. Well diffusion method

Agar well diffusion method is used for evaluating the antimicrobial activity of the compounds. Same amount 15-20 mL of Mueller-Hinton agar was poured on glass Petri plates of same size and allowed to solidify. Wells with a diameter of 8 mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. Standardized inoculums of the test organism (*Staphylococcus aureus*, *Escherichia coli*) were uniformly spread on the surface of these plates using sterile cotton swab. A volume (50  $\mu\text{L}$ ) of the extract solution at desired concentration was added to the wells and one well with Gentamycin maintained as positive and DMSO as a negative control. Then, the agar plates were incubated for  $37^\circ\text{C}$  for 24 hours for both

*Staphylococcus aureus* and *Escherichia coli*. After incubation, clear zone was observed. Inhibition of the bacterial growth was measured in mm.

### 4. RESULTS AND DISCUSSION

The current work discussed about the activity of antibacterial agents which depends on the nature of the metal ion present in the compounds. Generally complexes containing cobalt and copper metal ions exhibit high activity. In the present study even though the ligand had little effect on the bacteria their cobalt complexes exhibits significant potency. The effect of metal ions on the normal cell membrane defines the potency of metal complexes. According to chelation theory, metal chelates bear polar and nonpolar properties together makes them permeate through the cells and tissues which helps enhance or suppress the potential of the bioactive compounds.<sup>[11]</sup> This study reveals that the cobalt complexes of Benzilic acid –tyrosine ligand showed a significant potency against gram positive *Staphylococcus aureus* bacteria. It may be due to the cell wall structure of the bacteria is related to the antibacterial activity of the compounds. The membrane of the Gram-negative bacteria is surrounded by an outer membrane containing lipopolysaccharides. The non-lipophilic nature of our complex cannot restrict the growth of gram negative bacteria.<sup>[12]</sup> The activity of the compound depends on the concentration of the inhibitor, i.e; only at 800 mcg concentration the compound shows greater the inhibition. The activity of the compound was compared with the standard antibiotic Streptomycin. Four novel complexes synthesized and the ligand benzilic acid –tyrosine were tried against *Staphylococcus aureus*, *Escherichia coli*.

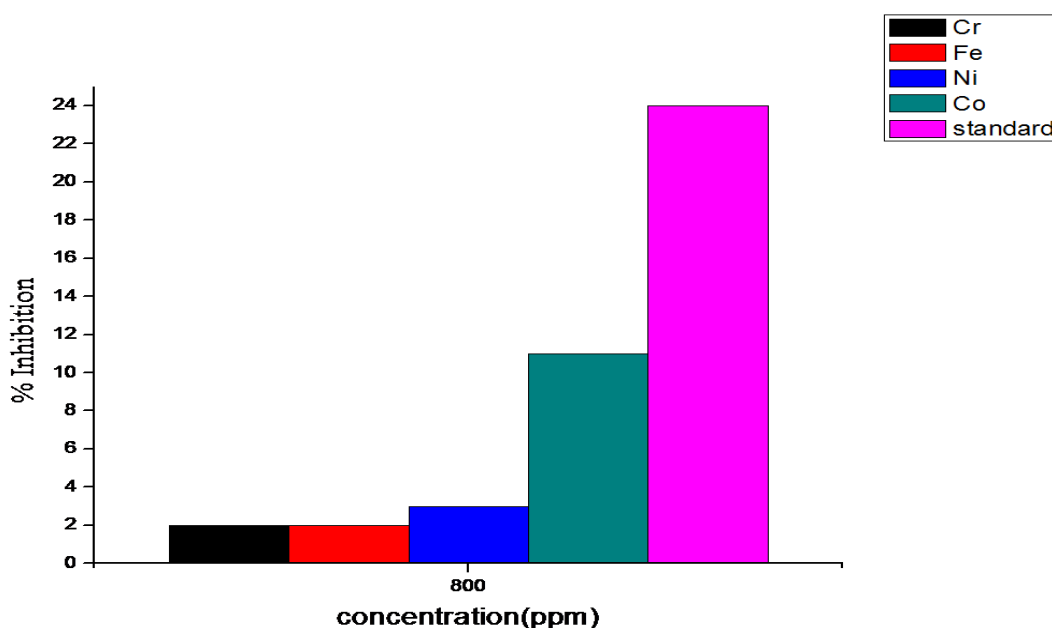


Fig 1: Benzilic acid tyrosine Cobalt complexes on *Staphylococcus Aureus*-48 hrs.

Table 2: Anti bacterial activity for different BT ligand and its metal complexes.

Name of the Pathogens	Antibacterial activity-Well diffusion method								Streptomycin (Positive control)	DMSO (Negative control)
	Zone of Inhibition (mm)									
	S1		S2,S3		S5		S4			
	400	800	400	800	400	800	400	800		
<i>Staphylococcus aureus</i>	-	-	-	2	-	3	2	11	24	-
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-	21	-

Fig.2. Comparative antibacterial study of cobalt complex of Benzilic acid-tyrosine ligand (S4) on gram negative *E. coli* bacteria and gram positive *Staphylococcus aureus* bacteria by using well diffusion method.

## 5. CONCLUSION

The new Benzilic acid-tyrosine ligand and its four metal complexes were synthesized and characterized with the aid of IR and electronic spectra. The pale yellow colored ligand changes its color on coordination with metal ion confirms the formation of the metal complexes. The octahedral nature of the complexes has been confirmed from the electronic spectra and magnetic measurements. The non-electrolyte nature of the complexes has been detected from the lower values of molar conductance. These compounds were not reported so far and this study helps for the preparation of such new compounds which are effective antimicrobial agents. The ligand which does not possess any activity against microbes enhanced its activity on complexation with the metal ion. Among the four complexes studied cobalt complex have the greater inhibition potency and the order of inhibition of the compounds is as follows: HBT < Fe (III)  $\approx$  Cr (III)  $\approx$  Ni (II) < Co (II). It is efficient against the gram positive bacteria *Staphylococcus Aureus* and do not have any action against gram negative bacteria. The Cobalt complex exhibits the inhibition action at a concentration of 800 ppm and above whereas it is inactive at lower concentrations.

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## 7. REFERENCES

1. S. Shanmukhappa and P. G. Ramappa, Asian journal of Chemistry, 1996; 8: 655-656.
2. WHO Report: Critically important antibacterial agents for human medicine for risk management strategies of non-human use, a report of a WHO working group consultation, Canberra, Australia., 15-18 February 2005.
3. Brit J., Pkarmacol. pharmacology of benzilic acid derivatives, 1951; 6: 634.
4. R. Sudha, Charles. C. Kanakam and G. Nithya, International Journal of Chem Tech Research, 2015; 5: 383-387.
5. G. Indiradevi, N. P. Pranamyia and M. Ali Hassan, World Journal of Pharmaceutical and Life Sciences, 2019; 5: 71-76.
6. G. Indiradevi, International Journal of Environment , Ecology, Family and Urban Studies, 2019; 9: 71-78.
7. Subhasish Saha, Dharumadurai, Dhanasekharan Saravanan Chandralekha Annamalai paneerselvam-Synthesis, Characterization and anti microbial activity of cobalt metal complex against multi drug resistant bacteria and fungal pathogen. series physics, chemistry and technology, 2009; 7: 73-80.
8. Nathmala and Yadav Rakesh, Bull.Chem.Soc, Jpn, 1997; 70(6): 1331-1337

9. Andreea Stanila , Cornelia Braicu , Sorin Stanila, Raluca M. Pop, Antibacterial Activity of Copper and Cobalt Amino Acids Complexes, Not Bot Horti Agrobo, 2011; 39(2): 124-129.
10. K. Nakamoto, P.J. McCarthy, 'Spectroscopy and Structure of Metal Chelate Compounds', John Wiley and sons, Inc, 1968; 289.
11. Nair MS, Arish D, Joseyphus RS. Synthesis, characterization, antifungal, antibacterial and DNA cleavage studies of some heterocyclic Schiff base metal complexes. J Saud Chem Soc, 2012.
12. Tumer M, Koksai H, Sener MK. Trans Met Chem 1999; 24: 414.